Immunohistochemical analysis of arterial wall cellular infiltration in Buerger's disease (endarteritis obliterans)

Masayoshi Kobayashi, MD, Masafumi Ito, MD, Atsuko Nakagawa, MD, Naomichi Nishikimi, MD, and **Yuji Nimura, MD,** *Nagoya, Japan*

Purpose: **The diagnosis of Buerger's disease has depended on clinical symptoms and angiographic findings, whereas pathologic findings are considered to be of secondary importance. Arteries from patients with Buerger's tissue were analyzed histologically, including immunophenotyping of the infiltrating cells, to elucidate the nature of Buerger's disease as a vasculitis.**

Methods: **Thirty-three specimens from nine patients, in whom Buerger's disease was diagnosed on the basis of our clinical and angiographic criteria between 1980 and 1995 at Nagoya University Hospital, were studied. Immunohistochemical studies were performed on paraffin-embedded tissue with a labeled streptoavidin-biotin method.**

Results: **The general architecture of vessel walls was well preserved regardless of the stage of disease, and cell infiltration was observed mainly in the thrombus and the intima. Among infiltrating cells, $CD3^+$ T cells greatly outnumbered $CD20^+$ B cells. $CD68^+$ macrophages or $S\text{-}100^+$ dendritic cells were detected, especially in the intima during acute and subacute stages. All cases except one showed infiltration by the human leukocyte antigen-D region (HLA-DR) antigen-bearing macrophages and dendritic cells in the intima. Immunoglobulins G, A, and M (IgG, IgA, IgM) and complement factors 3d and 4c (C3d, C4c) were deposited along the internal elastic lamina.**

Conclusion: **Buerger's disease is strictly an endarteritis that is introduced by T-cell mediated cellular immunity and by B-cell mediated humoral immunity associated with activation of macrophages or dendritic cells in the intima. (J Vasc Surg 1999;29:451-8.)**

Buerger's disease is a nonatherosclerotic, inflammatory, segmental vascular occlusive disease of unknown etiology, which involves medium and small arteries and veins of the limbs, only rarely affecting visceral or cerebral vessels. The disease was described and established in the English literature by Leo Buerger in 1908[1] as a clinicopathologic entity distinct from atherosclerosis. Today, Buerger's disease is accepted as a definite vascular disease with a typical clinical picture, natural history, and histopathology.[2-5] The diagnosis of Buerger's disease, however, remains controversial because of conflicting criteria used by many authors.[3-6] Pathologic diagnosis is considered of secondary importance.[2,4,7] Arteries from patients with Buerger's disease have not been analyzed with inflammatory infiltrating cell-specific monoclonal antibodies to discover whether cells of the immune system play a role in its pathogenesis.

Findings from nine patients, in whom Buerger's disease was diagnosed according to our criteria,[4,8,9] are summarized. The histologic and immunohistochemical features of Buerger's disease were described with monoclonal antibodies directed against surface antigens of T lymphocytes, B lymphocytes, macrophages, dendritic cells, and activated cells to identify the phenotypes of infiltrating cells and determine the distribution of various cell components in the arterial lesions.

MATERIALS AND METHODS

Case selection. Thirty-three specimens from nine patients, in whom Buerger's disease was diagnosed on the basis of our clinical and angiographic criteria (Table I) between 1980 and 1995 at Nagoya University Hospital, were studied. These materials were retrieved from the surgical pathology files of Nagoya University Hospital. Seven patients were

From the First Department of Surgery, Nagoya University School of Medicine, and the Department of Pathology (Drs Ito and Nakagawa), Nagoya University Hospital.
Reprint requests: Masafumi Ito, MD, Department of Pathology, Nagoya University Hospital, 65 Tsurumai-cho, Showa-ku, Nagoya 466, Japan.

0741-5214/99/$8.00 + 0 **24/1/94032**

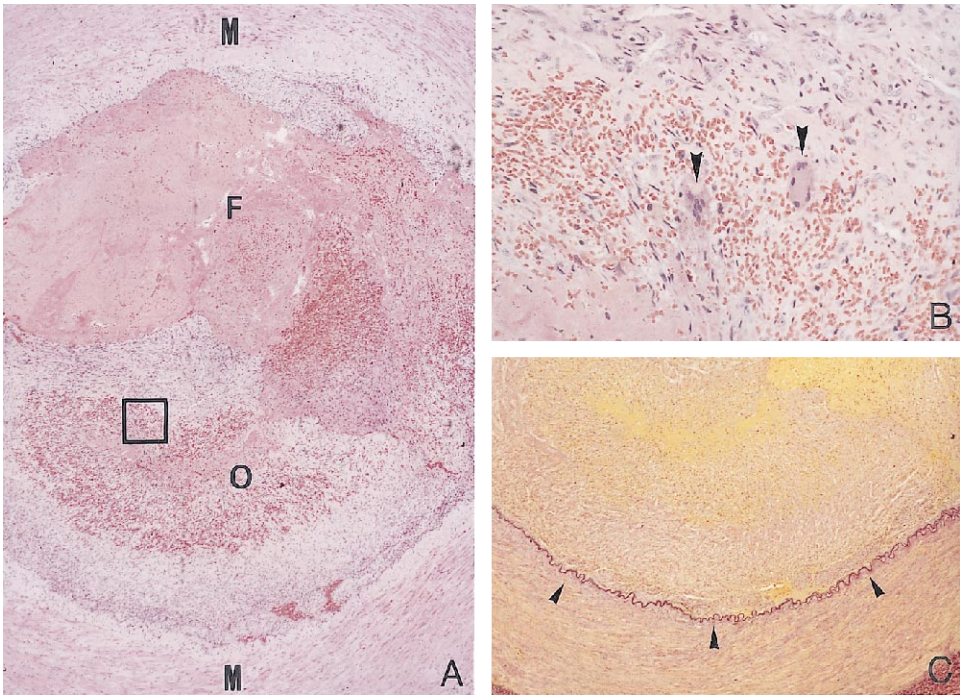

Fig 1. A, Typical acute lesion of the popliteal artery with thrombosis in Buerger's disease. (Original magnification, 40×; hematoxylin-eosin stain.) Fresh thrombus (*F*) and organizing thrombus (*O*) were seen in the lumen. There is a remarkable inflammatory cell infiltration in the intima and the organizing thrombus. Microabscesses and a multinucleated giant cell (*boxed area*) are observed in the organizing thrombus. *M,* Media. **B,** High-power view of boxed area in Fig 1, **A,** showing microabscess and multinucleated giant cell (*arrowhead*; original magnification, 400×; hematoxylin-eosin stain). These phagocytic giant cells are positive for CD68 and HLA-DR (not shown). **C,** The internal elastic lamina (*arrowhead*) is essentially intact in Buerger's disease (original magnification, 100×; elastica-van Gieson stain).

Table I. Clinical criteria for diagnosis of Buerger's disease

1	Smoking history
2	Onset before the age of 50
3	Infrapopliteal arterial occlusive lesions
4	Either upper-limb involvement or phlebitis migrans
5	Absence of atherosclerotic risk factors other than smoking

By Shionoya's criteria,[4] the clinical diagnosis of Buerger's disease is made only when all five requirements are met.

men, and two were women; the mean age of the patients at surgery was 43.4 years.

Light microscopic examination. All tissue was obtained from amputated limbs or other surgical specimens with informed consent from all patients. Paraffin-embedded tissue sections were stained with hematoxylin-eosin and elastica-van Gieson.

Immunohistochemistry. Immunohistochemical studies were performed on paraffin-embedded tissue by means of a labeled streptoavidin-biotin method. The primary antibodies used in this study, their specificities, and working dilution are listed in Table II. Endogenous peroxidase activity was blocked with 0.3% H_2O_2 in methanol for 30 minutes. For some antibodies, antigen expression was enhanced by means of pretreatment with either 0.5% trypsin digestion at 37°C for 30 minutes or heating in a microwave for 5 minutes twice in 0.1 mol/L citrate buffer at pH 6.0 (Table II). After incubation with primary antibodies overnight at 4°C, slides were incubated with biotinylated secondary antibodies (DAKO, A/S, Denmark) for 60 minutes and streptoavidin-peroxidase complex (Nichirei, Japan) for 60 minutes at room temperature. Color development used 3,3′-diaminobenzidine as chromogen with 0.005% hydrogen peroxide, followed by counterstaining with Mayer's hematoxylin.

Quantification of infiltrating cells on tissue sections. Each sample was viewed at 200× magnification high-power fields, and 20 randomly selected

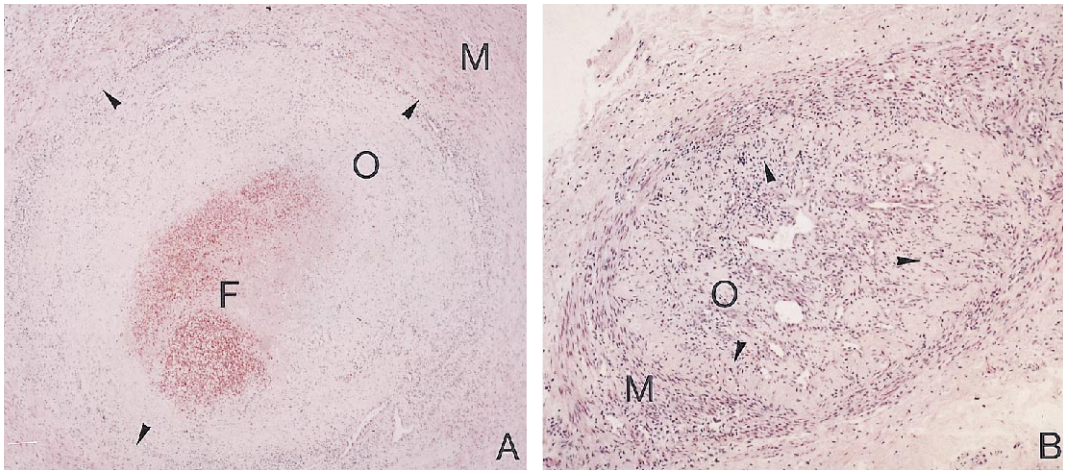

Fig 2. Photograph of subacute stage lesion (**A**) and chronic stage lesion (**B**). In the subacute lesion, fresh thrombi and organized thrombi are seen. A remarkable cell infiltration is observed in the thrombus and intima, however, it is scarce in the media (*M*). In the chronic lesion, the lumen is occluded by organized thrombi with recanalized vessels. Even in the chronic lesion, cell infiltration was observed. Note the internal elastic lamina is almost intact, regardless of the stage. *F,* fresh thrombus; *O,* organizing thrombus.

fields were analyzed in each section. In each case, the grades of positivity were as follows: –, negative in each field; 1+, 1% to 10% of the infiltrating cells positive per field at original magnification (100×); 2+, 11% to 20% positive; 3+, more than 20% positive.

Control samples. Arteries of three lower limbs affected with obliterative arteriosclerosis were used as controls.

RESULTS

Histologic findings. The nine cases examined are subdivided into one case of acute stage, two cases of subacute stage, and six cases of chronic stage, according to histologic criteria.[4,7,8] Case 1 showed a typical acute-stage lesion. The lumen was occluded by fresh thrombus and intimal thickening with remarkable infiltration of leukocytes, including neutrophils (Fig 1*A*). Multinucleated giant cells were seen (Fig 1*B*), but fibrinoid necrosis or granulomatous lesions were not observed. Two cases (case 2 and 3) had the histologic features of the subacute stage. These specimens had lumens occluded by fresh and organized thrombus with partial recanalization. Multinucleated giant cells were not found in thrombi or vessel walls (Fig 2*A*). Six cases had chronic-stage lesions. The occlusive thrombi were organized and recanalized extensively, and mild cell infiltration was seen in the intima, media, and adventitia (Fig 2*B*). The common features in all nine cases were general architecture and elastic laminae that remained essentially intact (Fig 1*C*) and cell infiltration that was observed predominantly in the thrombi and intima. No specimens revealed calcification and atheromatous plaque in the vessel walls.

Immunohistochemical findings. Immunohistochemical findings are summarized in Tables III and IV. There were more infiltrating cells in acute and subacute lesions than in chronic ones. Most of the infiltrating cells were seen in the intima, where $CD3^+$ T cells, which recognize all T lymphocytes, outnumbered $CD20^+$ B cells, which define all B lymphocytes, in ratios ranging from 2:1 to 5:1 in acute lesions (Fig 3*A*). Particularly in the chronic stage, $CD20^+$ B cells were more scarce than $CD3^+$ T cells. Though the $CD3^+$ T cells were seen mainly in the intima, some were scattered in the media and adventitia. In T lymphocyte subsets, $CD8^+$ T cells, which recognize the suppressor/inducer subset of T lymphocytes, were observed as often as $CD4^+$ T cells, which define the helper/inducer subset of T lymphocytes, in the acute stage (Fig 3*B*,*C*), unlike their normal ratio in blood. In the chronic stage, the number of $CD8^+$ cells predominated slightly over $CD4^+$ T cells.

$CD68^+$ cells, which define the macrophages, were observed particularly in thrombi and intima during the acute and subacute phases (Fig 4*A*). The population of $CD68^+$ cells was second in abundance,

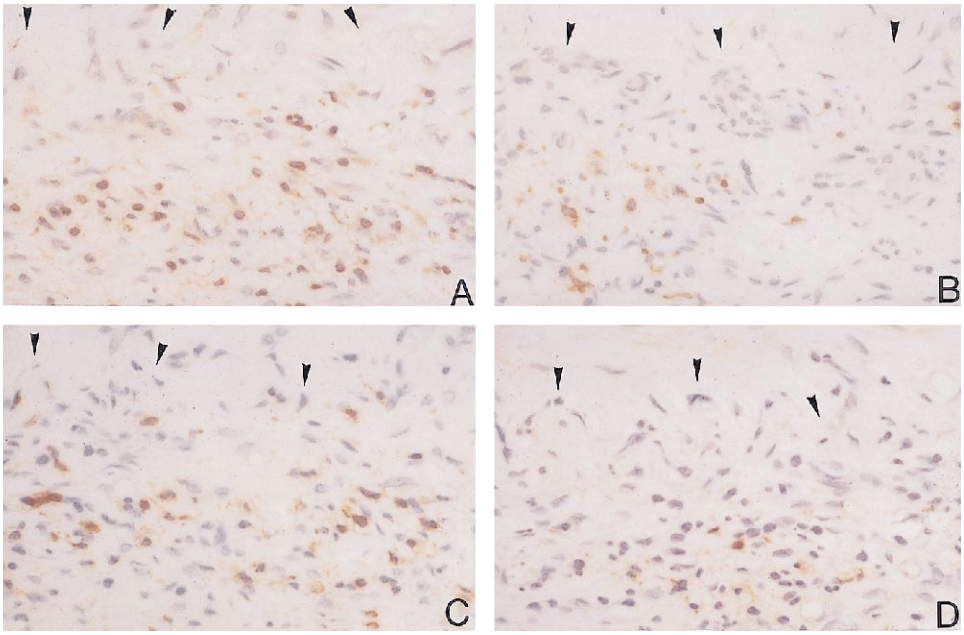

Fig 3. Cell infiltration along the internal elastic lamina (*arrowhead*), showing immunohistochemical staining for CD3 (**A**), CD8 (**B**), CD4 (**C**), and CD20 (**D**), respectively (brown cytoplasm; original magnification, 400×). The upper part, above the internal elastic lamina, is the media, and the lower part is the intima. Infiltrating cells are more obvious in the intimal layer than in the media. $CD3^+$ cells outnumbered $CD20^+$ cells in acute-stage lesions (**A, D**). $CD8^+$ T cells slightly predominate over $CD4^+$ T cells (**C, D**).

Table II. Antibodies and pretreatment used in this study

Antibody	*Specificity*	*Clone*	*Source*	*Dilution*	*Pretreatment*
CD3	Pan T cell		DAKO, Japan	× 150	0.5% trypsin
CD4	T helper/inducer cell	OPD4	DAKO, A/S, Denmark	× 100	
CD8	T suppressor/cytotoxic cell	DK25	DAKO, A/S, Denmark	× 100	Heating in a microwave
CD20	Pan B cell	L-26	DAKO, Japan	¥ 200	
CD68	Macrophage, monocyte	PG-M1	DAKO, Japan	× 100	
CD68	Macrophage, monocyte	KP-1	DAKO, Japan	× 150	0.5% trypsin
CD57	NK cell	Leu7	Becton-Dickinson	× 200	
CD34	Endothelial cell	QBEND/10	Seikagaku Corp.	× 80	
S-100	Dendritic cell		DAKO, Japan	× 500	
HLA-DR	Activation antigen	DK22	DAKO, Japan	× 50	
IgG	Gamma chain		DAKO, Japan	× 2400	0.5% trypsin
IgA	Alpha chain		DAKO, Japan	× 400	0.5% trypsin
IgM	Mu chain		DAKO, Japan	× 2400	0.5% trypsin
C3d complement	C3d		DAKO, A/S, Denmark	× 200	0.5% trypsin
C4c complement	C4c		DAKO, A/S, Denmark	× 200	0.5% trypsin

after the number of $CD3^+$ cells. $S\text{-}100^+$ cells, which recognize dendritic cells, were readily detected in the intimal layer (Fig 4*B*), but very few were detected in the media or adventitia in acute or subacute lesions. Particularly, $S\text{-}100^+$ dendritic cells in the thrombi and intima showed well-developed processes. More than 95% of $S\text{-}100^+$ cells showed the CD68 antigen simultaneously. Very few $S\text{-}100^+$ dendritic cells were recognized in chronic stage lesions. $CD57^+$ and $CD56^+$ natural-killer cells were observed scarcely in the thrombi and intima and media, however, not at all in the adventitia.

In all stages, especially acute and subacute lesions, $HLA\text{-}DR^+$ macrophages and dendritic cells were observed in the thrombi and intima (Fig 4). Giant cells in acute stage lesions expressed

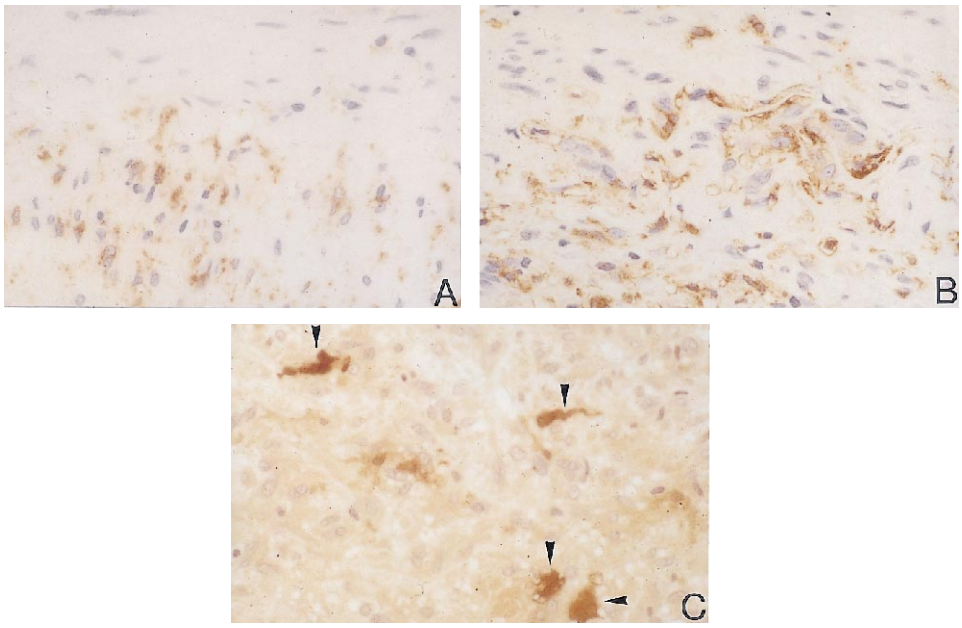

Fig 4. A, CD68+ cells are abundant in the intimal layer. **B,** HLA-DR+ cells are distributed in the intimal layer, along the internal elastic lamina. **C,** S-100+ cells (*arrowhead*) are found in the intimal layer in acute stage lesion. More than 95% of these S-100+ cells show the CD68 antigen simultaneously. Most of the CD68+ cells and S-100+ cells express HLA-DR antigen. (**A,** CD68; **B,** HLA-DR; **C,** S-100; original magnification, 400×.)

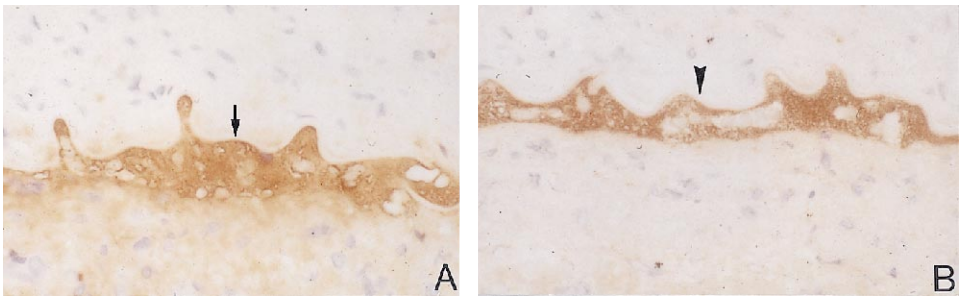

Fig 5. A, Deposition of IgG (*arrow*) and, **B,** complement factor 4 (C4c, *arrowhead*) along the internal elastic lamina, respectively. IgA, IgM, and C3d (not shown) were also deposited in the same fashion. (**A,** IgG; **B,** C4c; original magnification, 400×.)

HLA-DR and CD68 simultaneously, but never expressed S-100 on the cell.

IgG, IgA, and IgM were found to be deposited in a linear manner along the inner aspect of the internal elastic lamina (Fig 5*A*). Complement factors, C3d and C4c, were found to be deposited in a similar manner (Fig 5*B*).

The atherosclerotic control cases revealed fibrous intimal proliferation and hyaline degeneration and calcification in the media. The elastic lamina was severely reduplicated or fragmented or disrupted, in contrast with the intact elastic lamina in Buerger's disease. Leukocytic infiltration was observed in all three layers, however, its population was scarce (Fig 6). CD3+ cells were predominant, and among T lymphocytes, CD4+ T cells outnumbered CD8+ T cells. None of the three cases showed a linear distribution of IgG, IgA, IgM, C3c, and C4d along the area of elastic lamina, again in contrast with Buerger's disease. In the intima, HLA-DR+ cells were observed much less often than in Buerger's disease.

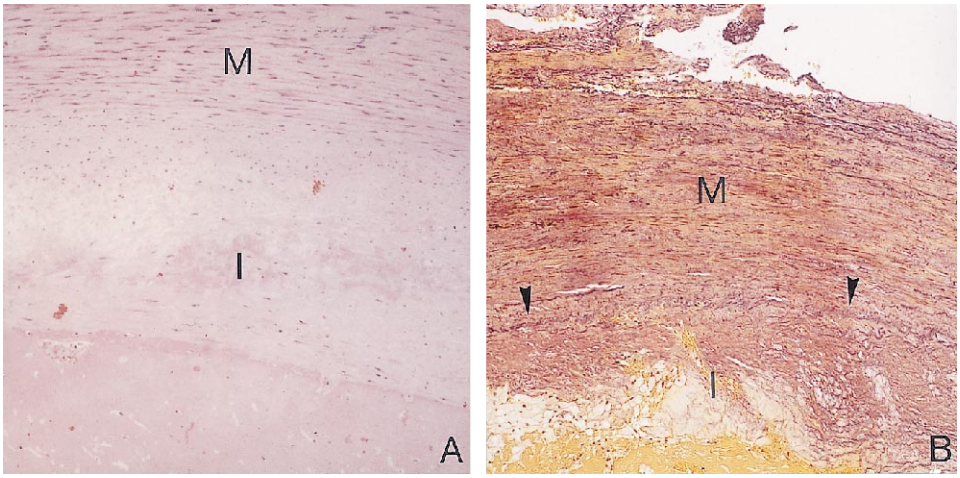

Fig 6. Photograph of atherosclerotic control cases. Fibrointimal proliferation and hyaline degeneration were seen (*I*). Foam cells were seen in the proliferated intima. Internal elastic lamina (*arrowhead*) and elastic fibers were found severely disrupted and fragmented. Cell infiltration were observed in all three layers, however, it was scarce. (**A,** Original magnification, 100×; hematoxylin-eosin stain; **B,** original magnification, 100×; elastica-van Gieson stain.) *I,* intima; *M,* media.

DISCUSSION

Buerger reported a disease of young men, in whom symptoms and signs of progressive vascular insufficiency led inevitably to gangrene and amputation. Buerger termed the disease "thromboangiitis obliterans" and distinguished it from atherosclerosis.[1] He considered acute inflammation and occlusive thrombosis of arteries and veins to be the characteristic lesions of the clinicopathologic entity.[10,11] In the long period since Buerger reported thromboangiitis obliterans, imprecise and conflicting clinical and pathologic criteria have caused much confusion and uncertainty in diagnosis. Tissue specimens seldom included the acute phase lesion, which Buerger viewed as essential for diagnosis.[1,11,12]

Chronic lesions, often said to be the least distinctive of the three stages of Buerger's disease, may be merely nonspecific residual evidence of arterial occlusion.[3,4,13] We examined all three stages by means of immunohistochemistry to determine which stage (acute, subacute, or chronic) possesses distinctive features.

Regardless of pathologic stage, the elastic lamina and all three layers of vessel walls were well preserved in Buerger's disease, in contrast with atherosclerosis. Additionally, certain findings readily distinguish Buerger's disease from other systemic vasculitides. In giant cell arteritis, the internal elastic lamina is characteristically disrupted and fragmented,[14,15] and in polyarteritis nodosa (classified as a necrotizing vasculitis), fibrinoid necrosis occurs in the vessel wall. Returning to comparisons with atherosclerosis, no calcification or hyaline degeneration were found in any cases of Buerger's disease, and cell infiltrates were more obvious in vessels affected by Buerger's disease.[16,17]

On the basis of our histologic observations, we believe that Buerger's disease is a vasculitis induced by an antigen in the intimal layer. In the acute case, some microabscesses were observed, indicating acute inflammation in the intima, which likely induced thrombus formation. Later, in the healing stage, various immunological phenomena appeared in the thrombus site. $CD4^+$ T lymphocytes are inducers of antibody production and regulators of cell-mediated immune responses. $CD8^+$ T lymphocytes display most of the cytotoxic activity and the suppressor for production of immunoglobulins. $CD8^+$ T cells may be involved in the initiation of the lesion, but $CD4^+$ T cells are at least as evident as $CD8^+$ T cells during progression of the lesions. $CD68^+$ macrophages and $S\text{-}100^+$ dendritic cells, thought to play a important role in the immune reaction,[18,19] were readily observed in the intima. Both HLA-DR–expressing macrophages and dendritic cells, as antigen-presenting cells in immune and inflammatory processes, were demonstrated mainly in the intima. In acute and subacute stages, macrophages and dendritic cells in the intimal layer appear to have been activated by an antigen. Such HLA-DR positive macrophages and dendritic cells may present an antigen from the intimal layer to T lymphocytes in Buerger's disease. In contrast, in

Table III. Results of immunohistochemical studies

		Antibody								
	Case number	*CD68*	*CD3*	*CD8*	*CD4*	*CD20*	*CD56*	*CD57*	*HLA-DR*	*S-100*
Intima	1	3+	3+	2+	2+	1+	1+	1+	3+	2+
	2	3+	3+	1+	2+	2+	1+	1+	3+	2+
	3	3+	3+	2+	1+	1+	1+	1+	3+	2+
	4	3+	3+	1+	2+	2+	1+	1+	3+	1+
	5	3+	3+	1+	1+	2+	1+	1+	1+	1+
	6	1+	2+	2+	1+	1+	1+	1+	–	–
	7	2+	2+	2+	1+	1+	–	–	1+	–
	8	2+	2+	2+	1+	1+	1+	1+	2+	1+
	9	1+	1+	2+	1+	1+	–	–	2+	1+
Media	1	1+	–	–	–	–	–	–	1+	1+
	2	–	–	1+	1+	–	–	–	1+	1+
	3	–	2+	–	–	–	–	–	1+	1+
	4	1+	2+	2+	2+	2+	1+	1+	1+	1+
	5	1+	1+	1+	1+	1+	–	–	–	–
	6	–	1+	1+	–	1+	–	–	–	–
	7	1+	1+	–	–	–	–	–	1+	–
	8	2+	2+	1+	2+	1+	–	–	1+	2+
	9	1+	1+	1+	–	–	–	–	2+	1+
Adventitia	1	1+	1+	–	1+	–	–	–	2+	1+
	2	2+	1+	1+	1+	–	–	–	1+	1+
	3	2+	2+	1+	1+	–	–	–	2+	1+
	4	1+	1+	1+	1+	–	–	–	1+	–
	5	1+	1+	–	1+	–	–	–	–	1+
	6	–	2+	1+	1+	1+	–	–	–	–
	7	1+	–	–	1+	1+	–	–	–	–
	8	2+	1+	–	1+	–	–	–	–	–
	9	1+	1+	1+	1+	–	–	–	1+	–

Case No. 1, acute stage; 2 and 3, subacute stage; 4–9, chronic stage.
–, negative; *1+*, 1%–10% positive of infiltrating cells; *2+*, 11%–20% positive; *3+*, more than 20% positive.

giant cell arteritis,[20] disease-related CD4+ T cells and dendritic cells are enriched in the adventitial layer, and interferon(IFN)-γ–secreting T cells accumulated in a circular fashion in the inner adventitia along the external elastic lamina. These findings also distinguish Buerger's disease from giant cell arteritis.

Both immunoglobulins and complement factors are deposited in a linear manner along the elastic lamina in Buerger's disease, which is characteristic of the acute or subacute phases. Although there are some reports that atherosclerotic plaques contain immunoglobulins deposits,[21,22] linear deposits similar to those in Buerger's disease were never found in the atherosclerotic arteries that we studied. Furthermore, in polyarteritis nodosa, deposits of immunoglobulins and complements are not consistently found within lesions, though circulating immune complexes are common.[23] These findings may imply that humoral immunity, which is induced by B cell activation, also plays an important role of immune reaction in the site of Buerger's disease. Usually, tissue damage caused by immunoreaction, such as vasculitis or glomerulonephritis, demonstrates deposition of not only immunoglobulins, but also complements. Finally, several unique, important observations in Buerger's disease are the infiltration of HLA-DR+ cells and CD68+ cells, and deposition of immunoglobulins and complement factors is linearly arranged along the inner aspect of an undisrupted internal elastic lamina.

Table IV. Deposition of immunoglobulins and complement factors along the elastic lamina

Case number	*IgG*	*IgA*	*IgM*	*C3d*	*C4c*
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
4	+	+	+	+	+
5	+	–	+	+	+
6	+	+	+	+	+
7	–	–	–	–	–
8	+	–	+	–	–
9	–	–	+	–	–

Case No. 1, acute stage; 2 and 3, subacute stage; 4–9, chronic stage.
+, Readily detectable; –, not detectable.

The identification of the inciting antigen in Buerger's disease remains unknown.[3,4] Smoking, infection, nutritional deficits, or general autoimmunity may be responsible for the antigen in Buerger's disease.[24,25] An as-yet-unidentified virus with or

without an intervening autoimmune process is another potential stimulus.

In conclusion, from our observations, it seems very likely that antigen presenting cells are activated by some unidentified antigen in the blood, resulting in an immunoreaction in Buerger's disease. An immune reaction (cellular, as well as humoral) is restricted to the arterial intima, which defines Buerger's disease as an endarteritis.

REFERENCES

1. Buerger L. Thromboangiitis obliterans: A study of the vascular lesions leading to presenile spontaneous gangrene. Am J Med Sci 1908;136:567-80.
2. Lie JT. The rise and fall and resurgence of thromboangiitis obliterans (Buerger's disease). Acta Pathol Jpn 1989;39(3): 153-7.
3. Papa MZ, Adar R. A critical look at thromboangiitis obliterans (Buerger's disease). In: Goldstone J, editor. Perspective in vascular surgery. Vol. 5, No.1. St. Louis: Quality Medical Publishing; 1992. p. 1-21.
4. Shionoya S. Pathology. In: Shionoya S, editor. Buerger's disease. Nagoya: The University of Nagoya Press; 1990. p. 57-79.
5. McKusick, VA. Buerger's disease: A distinct clinical and pathologic entity. JAMA 1962;181:93-100.
6. Adar R. Buerger's disease—The need for diagnostic criteria. Surgery 1974;76:848.
7. Lie JT. Diagnostic histopathology of major systemic and pulmonary vasculitic syndromes. Rheum Dis Clin North Am 1990;16:269-92.
8. Shionoya S. Buerger's disease (thromboangiitis obliterans). In: Rutherford RB, editor. Vascular surgery. 4th ed. Philadelphia: WB Saunders Company; 1995. p. 235-45.
9. Balas P, Faliakou E, Papalambros E. A case of Buerger's disease with pathology confirmation. Intern Angiology 1991;10:247-9.
10. Buerger L. Recent studies in the pathology of thrombo-angiitis obliterans. J Med Res 1914;26:181-94.
11. Buerger L. The pathological and clinical aspects of thromboangiitis obliterans. Am J Med Sci 1917;154:319-29.
12. Buerger L. Thromboangiitis obliterans: Concepts of pathogenesis and pathology. J Intern Chir 1939;4:399.
13. Lie JT. Thromboangiitis obliterans (Buerger's disease) revisited. Pathol Ann 1988;23:257-91.
14. Parums DV. The arteritides. Histopathology 1994;25:1-20.
15. Ashton-Key M, Gallagher PJ. Surgical pathology of cranial arteritis and polymyalgia rheumatica. Bailliere's Clinical Rheumatology Vol. 5, No. 3. 1991;387-404.
16. Gallagher PJ. Blood vessels. In: Sternberg SS, editor. Diagnostic surgical pathology. 2nd ed. New York: Raven Press; 1994. p. 1227-54.
17. Rosai J. Cardiovascular system. In: Rosai J, editor. Ackerman's surgical pathology. 8th ed. St. Louis: Mosby; 1995. p. 2194-216.
18. Vladimir R, Yurii VB, Galina KS, Irina K. Monocyte/macrophage accumulation and smooth muscle cell phenotypes in early atherosclerotic lesions of human aorta. Atherosclerosis 1993;100:237-48.
19. Yuri VB, Reginald SAL. S-100 positive cells in human arterial intima and in atherosclerotic lesions. Cardiovasc Research 1995;29:689-96.
20. Wagnere AD, Bjornsson J, Bartley GB, Goronzy JJ, Weyand CM. T cell activation in giant cell arteritis. Am J Pathol 1996;148:1925-33.
21. Hollander W, Colombo MA, Kirkpatric B, Paddock J. Soluble proteins in the human atherosclerotic plaque. Atherosclerosis 1979;38:391-405.
22. Hansson GK, Holm J, Kral JG. Accumulation of IgG and complement factor C3 in human arterial endothelium and atherosclerotic lesions. Acta Pathol Microbiol Immunol Scand 1984;92A:429-35.
23. Virmani R, Farb A, Burkle A. Systemic vasculitic syndromes. In: Virmani R, editor. Atlas of cardiovascular pathology. Philadelphia: WB Saunders Company; 1996. p. 164-77.
24. Gulati SM, Madhra K, Thusoo TK, Naif SK, Saha Kunal. Autoantibodies in thromboangiitis obliterans (Buerger's disease). Angiology 1982;33:642-51.
25. Roncon R, Delgado L, Correia P, Torrinha JF, Serrao D, Braga A. Circulating immune complexes in Buerger's disease. J Cardiovasc Surg 1989;30:821-5.

Submitted Jan 14, 1998; accepted Aug 18, 1998.